Establishing a Range-Wide Provenance Test in Valley Oak (*Quercus lobata* Née) at Two California Sites[1]

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Abstract

We present the methods used to establish a provenance test in valley oak, *Quercus lobata*. Nearly 11,000 acorns were planted and 88 percent of those germinated. The resulting seedlings were measured after 1 and 2 years of growth, and were outplanted in the field in the winter of 2014-2015. This test represents a long-term resource for both research and conservation.

Key words: Provenance tests, Quercus lobata, valley oak

Introduction

We set out to establish a long-term provenance test of valley oak (*Quercus lobata*) collected from across the species range. Provenance tests are designed to compare survival and growth (plus other morphological and phenological traits) among trees sampled from different parts of the species range (Mátyás 1996). By having different sources grown in a common environment, we are able to look at how different sources perform in a novel climate, and how they might respond to climate change (Aitken and others 2008). This study has a multitude of goals, from very practical management questions on how to source seeds for ecological restoration projects involving oaks, to very detailed ecological genomic studies. Our collecting and plant propagating methods were developed with a standard quantitative genetic analysis in mind (with plans for genomic analyses as well in the future).

Here we outline the methods used to establish a provenance test of *Quercus lobata* trees in California, and we present some very early results from the study.

Methods

We attempted to initiate this study in the fall of 2011; however, it was not a mast year, and acorns were difficult to find. We decided to collect from 10 field sites and use those acorns to establish a pilot study, with plans for the larger acorn collection in 2012. It turned out that 2012 had a massive acorn crop, and we were able to collect from 95 different sites from across California.

Overview

Acorns were collected and brought to the U.S. Department of Agriculture Forest Service (USDA FS), Institute of Forest Genetics (IFG), Placerville, California, where they were sown and then grown in the greenhouse for approximately 1 year. They were then stepped up to larger containers. On November 7, 2014, these trees were growing in two lath houses at the sites where they were to be outplanted in the winter of 2015: one at IFG, the other at the USDA FS Genetic Resource and Conservation Center (GRCC), Chico, California.

Acorn collection

Acorns were collected from across the species range in the fall of 2012, from October 9 to November 7. Sites were selected based on previous research, records of previous collections, and the species distribution across California with a goal to sample as broad an area of the species range as possible. Sites were separated by at least 30 km. There was a set of special collections done in three elevational transects: one at Mt. Hamilton, Santa Clara County, one outside of Madera into the Sierra Nevada foothills, and a third at Tejon Ranch in the Tehachapi mountains. These transects involved sampling trees along the elevational gradient at each of these sites.

At each of the 95 collecting sites, 20 acorns were collected from eight maternal trees (fewer if eight trees with acorns could not be found). The GPS location of each tree was taken, and a brief description of the site was written. A leaf sample for DNA extraction was taken from each tree. In addition, a herbarium sample was collected from each tree, consisting of a small branch with flowers and leaves. The DNA sample was kept cool until it could be placed in a -80 °C freezer. The herbarium sample was pressed in the field. The DNA and herbarium samples were returned to the Sork Lab at the University of California, Los Angeles (UCLA). The acorns were transported to IFG.

After collection, the acorns were stored in a walk-in cold box at 1.1 °C in paper bags inside unsealed zip lock plastic bags to keep the acorns moist, but to allow air flow to prevent rot.

Acorn planting

On November 14 and 15, 2012, 10,326 acorns were planted into Stuewe and Sons D40 containers, 6.4 cm diameter, 25.0 cm depth, 656 ml volume, 20 cells per rack. This container was selected because it had adequate volume for the first year growth and we were able to fit the amount of racks needed to sow the approximately 10,000 acorns into our greenhouse. We used Sunshine #4 aggregate plus soil mix. This mix contained Canadian sphagnum peat moss, coarse perlite, starter nutrient charge (with gypsum), dolomitic limestone, and long-lasting wetting agent. This soil was selected because it is an aggregate mix and, unlike vermiculite mixes, the soil holds its form when plants dry down, giving more flexibility with watering. The aggregate is preferred when growing a large number of plants to alleviate the slower growing plants from being too wet. The acorns were germinated under a 50 percent shade cloth.

With such a large amount of acorns to plant, we balanced our sowing layout between experimental design and practical culturing. Each family (here family = a collection from a single maternal tree) was given an accession number to simplify the

identification of each tree and to lessen the amount of error. The planting of the eight families (accessions) from each of the 95 collection sites was randomized throughout the greenhouse, but the 16 acorns planted within each family were sown in the same rack. This balance was made to reduce the error factor as we progressed with this study.

All collections were then randomized for sowing. There are distinct differences of growing conditions in the greenhouse and the location of each family was mapped within the greenhouse to allow for future data analyses to take that location information into account.

Each seed lot of acorns was surface sterilized with a 10 percent bleach solution to remove any molds or contaminants. The acorns were submerged in the bleach solution for one minute then rinsed in clean water before planting. The bleach solution was changed periodically during the planting process (fig. 1a). Acorns that floated were removed from the experiment. Acorns for each family were bulked weighed, and the number of acorns planted per family was recorded, allowing for the calculation of an average seed weight for each accession.

At planting, the acorns were embedded in the soil sideways but not covered; this prevented the acorns from being planted upside down and mimicked what happens in nature (fig. 1b).
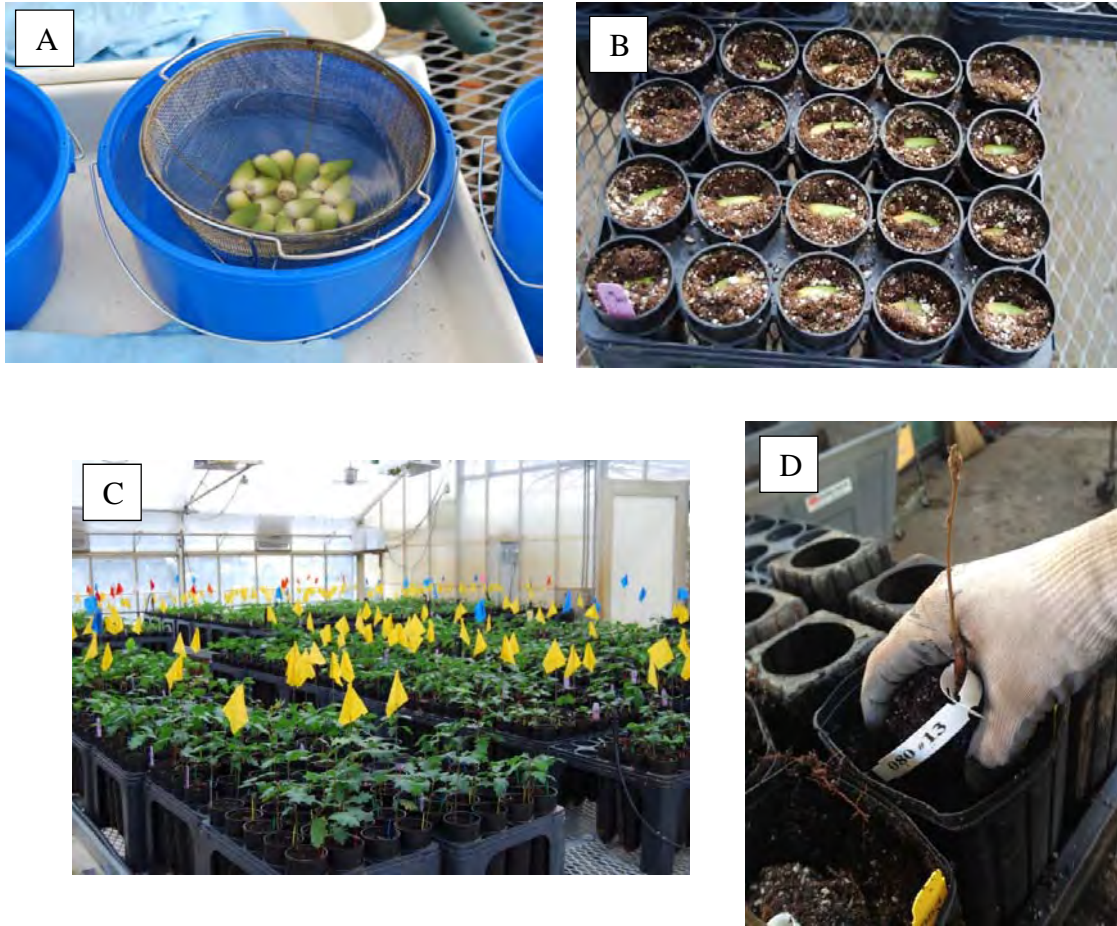
Figure 1—Photographs from the planting of the acorns. A. the bleaching process. B. the sowing of the acorns. C. recording germination in a crowded greenhouse. D. Stepping up trees to larger pots.

It was determined in the 2011 pilot study that the soil and container were appropriate. In the pilot study, however, the greenhouse was kept at warmer growing temperatures which resulted in erratic germination, making it difficult to properly culture the growing plants among the non-germinated ones. We also felt that the unnatural germination time could influence our growth measurements. Therefore, for the study, the greenhouse was kept cool in the daylight hours (approximately 16 °C) and cold at night (approximately 3 °C). This was done to mimic what would be happening in nature and to achieve more uniform germination. It was observed that the acorns put down a tap root but did not put out any leaves. The greenhouse was kept cold through the winter months and warmed up with the ambient outside temperatures when the acorns started to naturally put on top growth. This worked very well for achieving uniform germination throughout the greenhouse. Acorns were germinated under a 50 percent shade cloth.

The soil moisture level was closely monitored and soil was kept moist but not wet. Until the leaves started to appear, the tubes were kept at the proper moisture level with mist sprinklers, set to mist twice a week. When leaves started to appear the

tubes were hand watered and adjustments were made in amount of water depending on the dryness of the soil in each rack. Where there were dry spots, those racks were watered twice; each container was filled with water, allowed to drain, and filled again.

Mildew was a problem, despite reducing humidity by watering in the early hours and opening the 50 percent shade cloth, giving the plants full sunlight for the majority of the day. The mildew level was unacceptable and the plants were sprayed with Eagle® 20EW fungicide at a rate of 35.5 ml per 99.2 m^2 (1.2 fl oz per 1000 sq ft) containing Activator 90®, 5 ml /3.78 l (1 tsp/gal) as the surfactant. This was done twice (May 17, 2013 and May 16, 2014). The only other pest was aphids, which appeared at the end of the growing season when the trees where shutting down and the leaves where starting to senesce. The IFG greenhouse practice is to limit the application of chemicals, especially insecticides. To achieve an acceptable balance of beneficial insects and aphids, a certain amount of insects must be tolerated. The protocol of removing infected leaves and keeping the greenhouse tidy of leaf debris is our common practice and was effective at keeping the aphids at an acceptable level.

Germination

Germination data was taken weekly using printed pot stakes starting January 2, 2013 and continuing to April 29, 2013. The number of days until germination was calculated on the Monday of each week. We recorded all the germination data from the printed tags when the growth measurements were taken. Germination was scored as above-ground emergence, since the tap root germination was impossible to monitor. As more germination occurred the ability to observe new germinants was difficult, so a flag was put into each tube with an acorn that had not begun to put on leaves to aid in detecting the later germination (fig, 1c). When the majority of germination was achieved, weekly monitoring of germination was discontinued and all new seedlings were put into the category of "late germination" (after 182 days). At this point the trees within a family (accession) were given individual identification numbers becoming individuals within each family.

There was an observed greenhouse location effect on germination. To ensure those effects were captured in data analysis, the greenhouse location of each accession was mapped and recorded. The greenhouse areas were as follows: Bay one-South window, South aisle, North window, North aisle; Bay two- North window North aisle.

Mutants

When growing a large number of individuals of one species it is common to see a number of mutations and other odd growing forms manifest in the phenotypes (fig. 2). We observed a number of albinos, and tissue was collected from each albino for future genetic analyses. There were other mutations as well and some of these plants died. Other phenotypes were temporary, and the plants later developed normal appearing leaves. These plants were taken out of the study but kept for observation. Some of the surviving mutants will be planted at the IFG site for future observation.

Figure 2—A set of mutant plants observed in this study.

Selection of 12 best plants

The best 12 of the 16 plants were selected to continue growing for the study. The culled trees were removed from the greenhouse beginning in July of 2013. Their germination date was recorded, and they were measured for height and diameter shortly after culling, a few months before the rest of the trees were measured. Therefore their height data was not compared directly with the main experimental trees. However, the change in basal diameter in that time was expected to be minimal.

First year measurements

Growth measurements were taken on all experimental seedlings in September and October of 2013. A total of 9,129 seedlings were measured. Measurements were disrupted by the government shutdown of 2013; however, temperatures were fairly cold at that point and only minimal additional growth was observed. Three leaves were collected from each seedling for further morphological analyses. We took height and diameter measurements for each plant using a meter stick and calipers.

Stepping up into larger pots for second year growth

To allow for more root growth, seedlings were repotted into larger pots in the winter of 2013-2014 (fig. 1d). We selected Stuewe and Sons TP414 pots—10.0 cm wide, 36.0 cm high, volume 2.83 liters. The same soil that was used in the germination phase was again used. We added Osmcote® 14-14-14 at a rate of 0.45 kg per 0.1 m^3 (1 lb per 3.8 cu ft), mixed into the soil when filling the pots.

When the plants were stepped up to the larger pots, they were also moved into their complete randomized block design, as they will be planted into in the field. This was done to help with the organization and logistics of planting into the ground and will help account for the lath house location effects in the second year of growth. The field planting design consists of each maternal tree being represented once in each block, with the maternal trees from a site grouped together in a row-plot. This is a traditional design for provenance tests (Conkle 1973, Kitzmiller 2004) and allows for easier and more effective maintenance of the test over time. The trees will need to be thinned in the next 5 to 10 years, and this design facilitates that process. The position of each of the row-plots is also randomized between the blocks.

At this point the trees were separated into two groups, one for each of the planting sites, IFG and GRCC. The trees to be planted at the GRCC in Chico were then moved to a lath house at the planting site (fig. 3a, c). The trees remaining at IFG were moved to a lath house at the site (fig. 3b). This was done so the plants would acclimate to their eventual planting location. The plants where placed outside on benches that we are able to forklift out to the field. Figure 4 shows a Principal Component Analysis (PCA) of climate data derived from each of the collecting sites using the USDA FS climate estimates from Crookston and Rehfeldt (2008). The two planting sites are shown in white. The figure illustrates two points. First, it shows that the two planting sites both fall within the range of climate variation observed across all of our collecting sites. Second, the two planting sites are in fact quite different in terms of climate.

Figure 3—Photos from the lath house phase of the study. A. Moving the trees to the GRCC in Chico. B. The IFG lath house. C. The GRCC lath house.
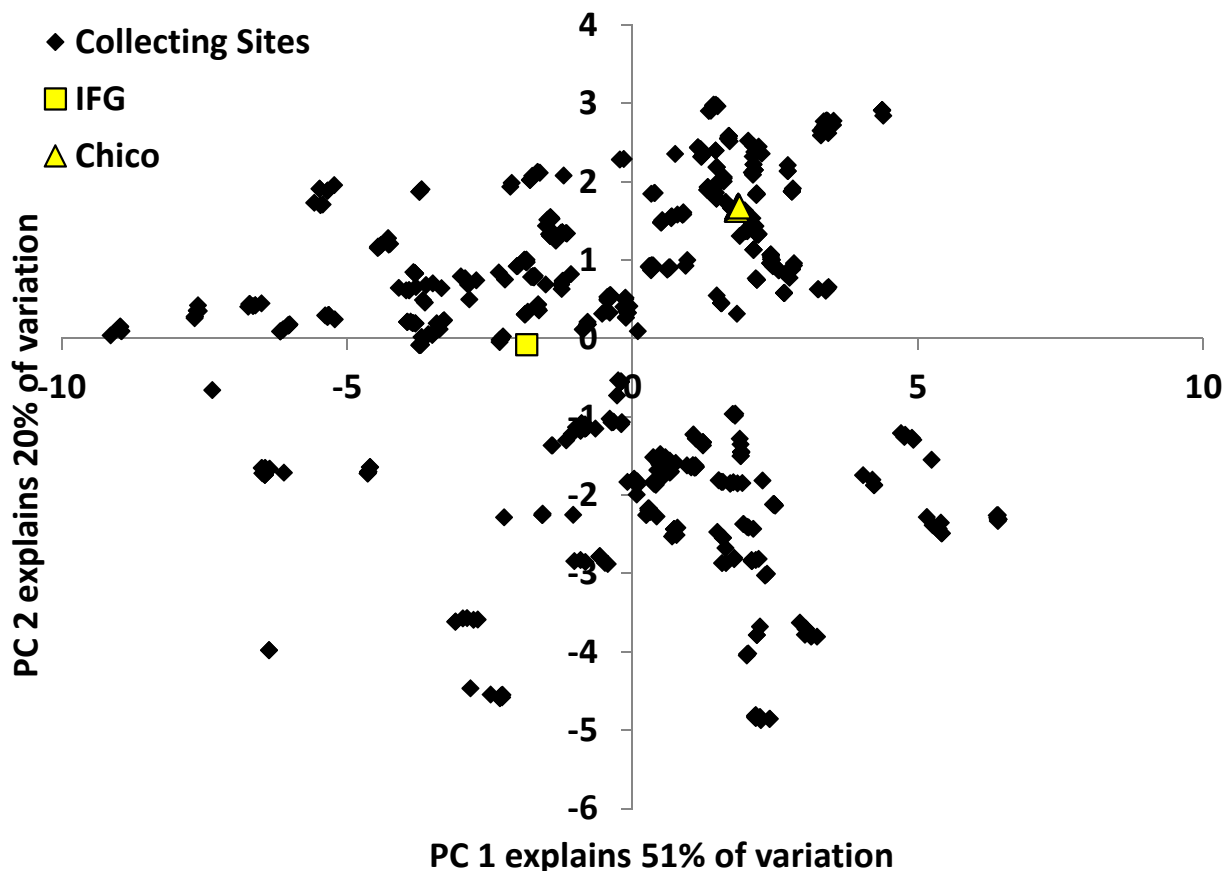
Figure 4—A graph showing the results of a PCA of climate data derived for each of the acorn collecting sites and the two planting sites.

At the end of their second growing season, total height and basal diameter were again recorded for each plant in the experiment, and the extra plants that will be part of the field boarder rows.

Results

Germination

Despite keeping the greenhouse cool, some above-ground germination started in the winter months. We began taking germination records to capture this early germination at 49 days after sowing, but the majority of the germinations happened between 61 and 103 days after sowing (fig 5). Overall, we observed an 88 percent germination rate.
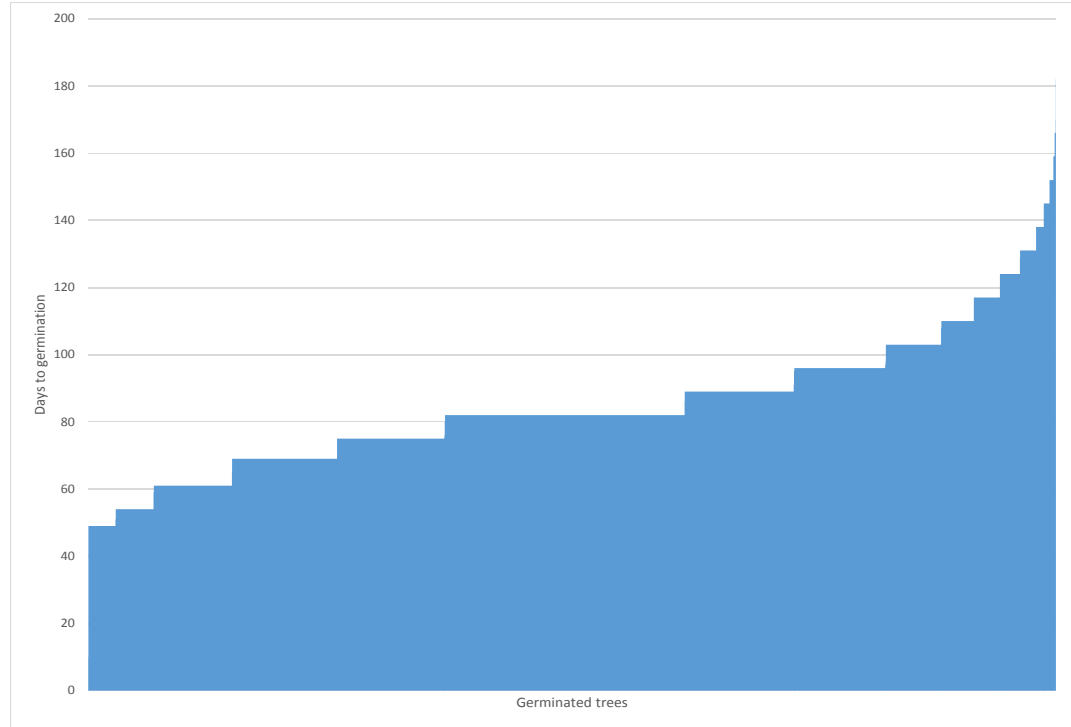
Figure 5—Chart showing the observed germination date for all 9110 individuals that germinated in this experiment.

First year measurements

Analysis of genetic correlations (correlations using the family mean values) among the measured traits showed that germination date was negatively correlated with all of the other measured traits (germination rate, acorn weight, basal diameter and height) (fig. 6a). Acorn weight was positively correlated with all measured traits except germination date (heavier acorns germinated earlier) (fig. 6a).

Comparing performance between benches, we did observe variation across the greenhouse in the growth of trees (fig. 6b). This suggests that indeed we need to keep track of the trees first planting location in future analyses. We will also be able to track this effect over time. We expect it to be random with respect to the future planting of the experiment because the planting location was randomized with respect to where the seedlings were growing in the greenhouse.
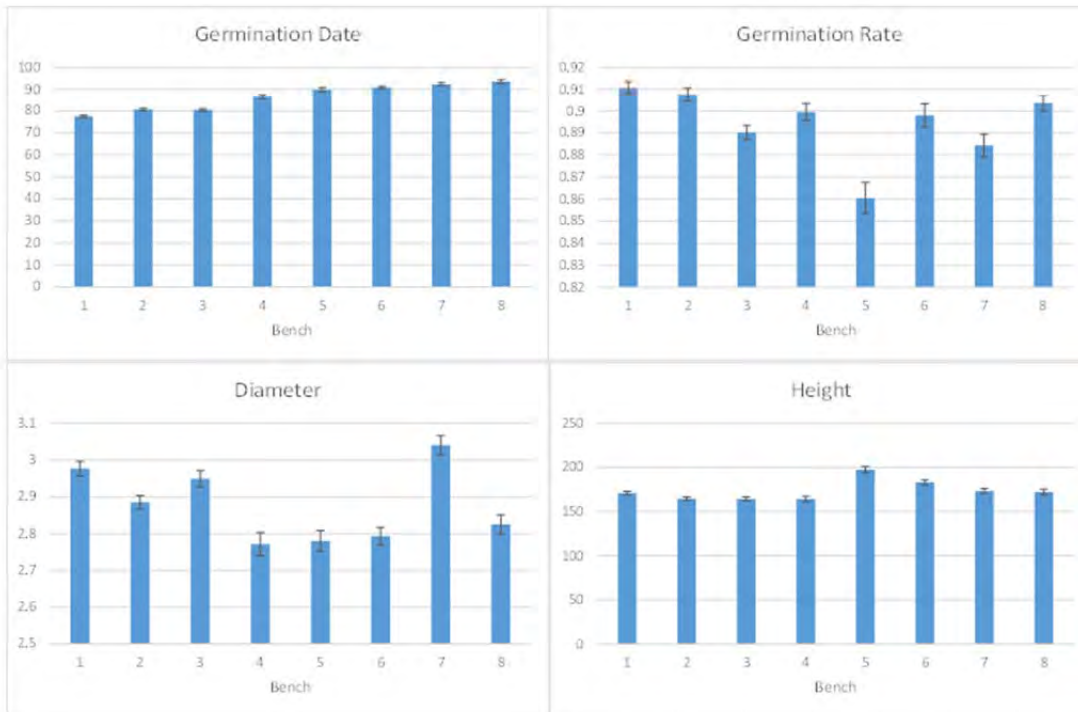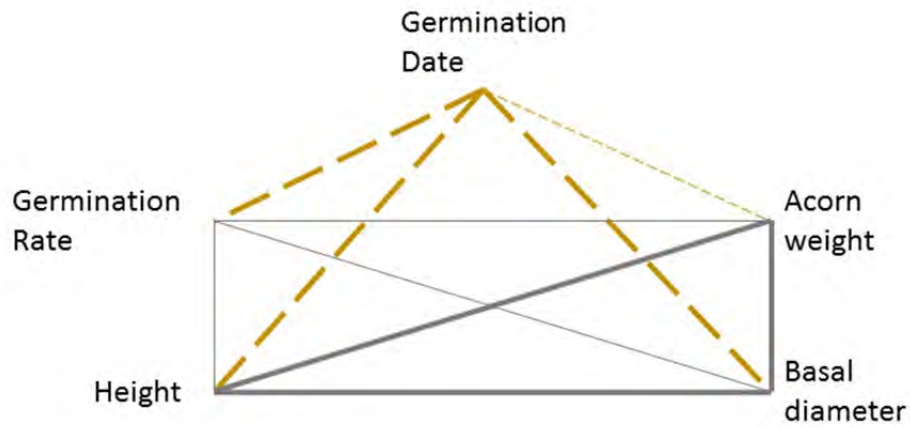
Figure 6—a. Correlations among the first year growth traits. Solid lines show positive correlations, dashed lines show negative ones. The thinner lines are significant at p <0.05. The thicker lines are significant at p <0.0001. b. Differences among the eight greenhouse benches where the seedlings were growing when they were measured after 1 year of growth.

Discussion

Valley oaks are under threat due to land conversion, and more recently, climate change. Information is lacking on the ecological genetics of this species, information that could be key to our understanding of how to manage this species as climate changes.

Here, we show that there are genetic correlations among traits and that there was variation in the timing of germination among these acorns collected across the species range. These early results are not appropriate to use for management decisions; however, they do indicate that this study is poised to produce results that will inform future management of oak woodlands.

This study represents a long-term investment to gain knowledge of the ecological genetics of this species. Once out-planted in the winter of 2014-2015, we will continue to collect survival and growth data for the next 20 to 50 years. In addition, we plan to collect additional morphological data on leaf traits as well as phenological data-recording the date when buds start to expand in the spring.

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